Interruption of Semiochemical-Mediated Attraction of *Dendroctonus valens* (Coleoptera: Scolytidae) and Selected Nontarget Insects by Verbenone

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ABSTRACT We tested two formulations of verbenone for efficacy in protecting ponderosa pine trees from attack by the red turpentine beetle, *Dendroctonus valens* LeConte: (1) polyethylene bubblecaps filled with 800 mg of neat verbenone, and (2) a sprayable water suspension of microencapsulated verbenone. We baited artificial trees (cardboard cylinders coupled with Lindgren pheromone traps) with host kairomones and the verbenone formulations. Efficacy was measured by numbers of beetles captured in baited traps. Both release systems significantly reduced trap catch, and there was no significant difference between them. Both systems have promise for use in forest stands, depending on management objectives and land use. Verbenone was an interruptant for some nontarget insects, especially Coleoptera, and none were consistently attracted to verbenone. We report on verbenone as an interruptant to host attraction in the red turpentine beetle, and also on the efficacy of a microencapsulated scolytid semiochemical.

KEY WORDS red turpentine beetle, *Pinus ponderosa,* plantations, semiochemicals, microencapsulation, *Leptura obliterata*

THE RED TURPENTINE beetle, *Dendroctonus valens* LeConte, is a widespread but usually minor pest of all pine species within its range in the United States, Mexico, and Canada (Bright 1976, Furniss and Carolin 1977, Cibrian Tovar et al. 1995). In addition, the beetle was recently introduced to China (Sun Jianghua, Chinese Academy of Sciences, Beijing, personal communication). Attacks by *D. valens* frequently occur on injured or stressed trees (Smith 1961). In ponderosa pine, *Pinus ponderosa* Laws (Pinaceae) stands with a high incidence of black stain root disease [*Leptographium wagnerii* variety *ponderosum* (Harrington & Cobb), Harrington & Cobb] attacks by *D. valens* proved to be an indicator of disease and impending tree mortality (Owen 1985). *D. valens* was recently found to be the cause of appreciable mortality in a thinned, subsoiled ponderosa pine plantation near Pondosa, CA. In China, it is causing severe mortality in *Pinus tabuliformis* Carrière plantations (Sun Jianghua, Chinese Academy of Sciences, Beijing, personal communication). Owen et al. (1987) found that *Leptographium terebrantis* Barras & Perry, a bluestain fungus carried by *D. valens,* was the most virulent of a group of fungi isolated from *D. valens* and congeners. This pathogenic fungus could be an important factor contributing to tree mortality in *P. ponderosa.*

Although insecticide applications to the basal portions of tree boles have been shown to be effective in reducing damage by *D. valens* (Hall 1984, Svirha 1995), in many situations a more environmentally benign method of control, such as the use of semiochemicals, is preferable. The semiochemical (S)-(-)-verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) has been shown to be effective in interrupting the response of other *Dendroctonus* Erichson spp., including *Dendroctonus terebrans* (Olivier), *Dendroctonus ponderosae* Hopkins, *Dendroctonus adjunctus* Blandford, *Dendroctonus brevicomis* LeConte, and *Dendroctonus frontalis* Zimmerman, to conspecific pheromones, host volatiles, or stressed host trees (Borden 1982, Livingston et al. 1983, Payne and Billings 1989, Phillips et al. 1989, Paine and Hanlon 1991, Bertram and Paine 1994, Miller et al. 1995). We hypothesized, therefore, that verbenone would also interrupt host attraction in *D. valens.* Bark beetles in the family Scolytidae are considered very good candidates for the development of semiochemical-based control strategies (Borden 1997). To that end, we initiated a study to test two release systems of verbenone for protection of *P. ponderosa* plantations from damage caused by *D. valens.* One of the release systems, polyethylene bubblecaps (Phero Tech, Delta, British Columbia), has been tested and proven effective in controlling other *Dendroctonus* spp. (Miller et al. 1995, Ross and Daterman 1995). The other system, a sprayable water suspension of microencapsulated verbenone (3M, London, Ontario), is a novel product that has not, to our knowledge, been tested for efficacy against forest scolytids.

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Although our primary focus was on the behavioral response of *D. valens,* we also tallied responses of nontarget insects, because it is ultimately important to anticipate unintended effects on predators, parasites, and associated herbivores (Raffa and Dahlsten 1995, Aukema et al. 2000).

Materials and Methods

Field Site. Our study was conducted in a 17-yr-old, 2,266 ha ponderosa pine plantation in Siskiyou County near Pondosa, CA (41° 11.9732′ N, 121° 40.2658′ W, elevation 1,231 m). This plantation was created at the site of the Pondosa fire of 1982, which was an extensive, stand-replacing fire. Soil had been compacted during salvage logging and was further compacted when the new plantation was mechanically thinned in 1998. After thinning, traffic lanes were subsoiled in some parts of the plantation to rehabilitate the twice-compacted soils. Some pine roots were damaged in the subsoiling process. The combination of thinning and subsoiling was associated with *D. valens* attack. Tree mortality from *D. valens* attack became evident in 1999 and continued in 2000. We estimated that, in thinned plots, ≈15% of standing trees had been killed by *D. valens.*

Assay of Verbenone Formulations. We chose to install surrogate trees (cardboard cylinders, 24.0 cm diameter × 90.3 cm long, Pacific Paper Tube, Oakland, CA) instead of using existing live trees for three reasons. First, the use of real trees could be expected to result in higher variance (more "noise") because pine monoterpene composition is known to be extremely variable (Smith 1977), beetle attack further increases this variability (Wallin and Raffa 1999), and variations in monoterpene release are also known to affect beetle response (Siegfried et al. 1986). Second, our baits were designed to attract *D. valens* in numbers sufficient to kill young plantation trees, and we chose not to risk increasing tree mortality in the plantation until we had assessed efficacy of the formulations in inhibiting beetle response to surrogate trees. Finally, we wanted a consistent physical substrate for release of the microencapsulated formulation, and the cylinders were superior in this respect to real trees. We deployed the cylinders in all treatments, including those where the treatment was a bubblecap rather than a spray, to standardize treatments and avoid bias in beetle response to visual stimuli.

Thirty surrogate trees were placed 20 m apart in a grid, and treatments (10 replicates for each of three treatments) were randomly assigned to trees (Table 1). An 8-funnel Lindgren trap (Phero Tech, Delta, British Columbia) baited with a *D. valens* kairomone lure (Phero Tech) was suspended just above the ground next to each surrogate tree to challenge the repellency of the two verbenone release systems. Collection cups on the Lindgren traps were filled to 2.5 cm with propylene glycol as a killing agent. The first verbenone release system consisted of polyethylene bubblecaps filled with 800 mg neat verbenone with 1% UV stabilizer (Phero Tech) and the second consisted of a sprayable water suspension of microencapsulated verbenone, hereafter referred to as the MEC (3M, London, Ontario). The full length of the cardboard tubes was sprayed to run-off with the MEC using a hand-held garden sprayer (RL Flo-Master, Root-Lowell Manufacturing, Lowell, MI). Untreated cylinders were placed adjacent to kairomone-baited Lindgren traps for both the controls and the verbenone bubblecap-baited treatments, to avoid confounding beetle responses to a tree-shaped visual cue. Trap collection cups contained propylene glycol as a killing agent. The study was installed on 31 July 2000, and traps were left in place until 17 August 2000 (18 d later) when insects were collected and target and nontarget responses tallied. Voucher specimens have been deposited in the Essig Museum of Entomology at the University of California, Berkeley.

Table 1. Treatment description

Treatment	Attractive bait	Interruptant
Control	*D. valens* kairomone	None
Bubblecap	*D. valens* kairomone	Verbenone bubblecaps attached to Lindgren funnel
MEC	*D. valens* kairomone	Verbenone MEC sprayed on surrogate tree

D. valens kairomone = combination of (−)-β-pinene, (+)-α-pinene, and (+)-3-carene in a 1:1:1 ratio; chemical purity of verbenone in bubblecaps = >97%, enantiomeric ratio (S:R) = 83:17; chemical purity of verbenone in MEC = 98%, enantiomeric ratio (S:R) = 97:3; MEC = microencapsulated formulation.

A preliminary midseason test of the verbenone bubblecap treatment was conducted between 28 June and 31 July 2000, before the MEC formulation was available. That test used the experimental design described above, except that treatments destined to receive MEC applications were used as an extra set of controls. Ultimately, a greater diversity of nontarget insects responded in the earlier test than in the late season test, so we report those results below.

Experimental Design and Statistical Analysis. Treatments were replicated 10 times in a completely randomized design. Trap counts were analyzed with generalized linear models for over-dispersed Poisson distributed responses (counts) (McCullough and Nelder 1989). Results are tabulated as estimated means, with 95% confidence intervals of the true means as a measure of spread around the estimates of the means. Multiple comparisons were based on the maximum likelihood ratio test with the Bonferroni approach (experiment-wise $\alpha = 0.05$). SAS GENMOD procedures (SAS Institute 1997) were used for the analysis.

Results and Discussion

Verbenone interrupted the response of *D. valens* to the host kairomone blend in both tests (Tables 2 and 3). This finding is in accordance with its effect on several congeners, including *D. terebrans, D. brevicomis, D. ponderosae, D. adjunctus,* and *D. frontalis* (Livingston et al. 1983, Payne and Billings 1989, Phil-

Table 2. Midseason response of *Dendroctonus valens* and nontarget insects to traps baited with host kairomones, with and without verbenone bubblecaps

Trt	*D. valens*	Cerambycidae	Buprestidae	Elateridae	Cleridae	Raphidiidae	Siricidae
CTR	3.8a (2.5–5.4)	1.9a (1.3–2.6)	0.5a (0.3–0.9)	2.5a (1.6–3.9)	0.8a (0.4–1.3)	0.9a (0.4–1.7)	0.2a (0.1–0.5)
BCP	0.1b (0.0–2.6)	1.4b (0.8–2.4)	0.1b (0.0–0.7)	0.5b (0.4–2.9)	0.1b (0.0–0.9)	0.7a (0.2–2.0)	0.4b (0.2–1.0)

Mean number of insects responding per trap (lower and upper 95% CI); means followed by different letters in a column are significantly different at $\alpha = 0.05$, maximum likelihood test with Bonferroni approach; CTR = Lindgren traps baited with host kairomones (control); BCP = Lindgren traps baited with host kairomones and verbenone bubblecaps.

lips et al. 1989, Paine and Hanlon 1991, Miller et al. 1995). In the midseason test using just the bubblecap treatment, verbenone reduced trap catch from a mean of 3.8 beetles/trap to 0.1 beetles/trap, a reduction of 99.3% (Table 2). In the late season test using both formulations, trap catch was reduced 82.1% by the bubblecap formulation and 89.3% by the MEC formulation (Table 3). This finding is important because effective new methods of control, especially EPA-registered biopesticides, are increasingly desirable. In the late season test, both of the release systems trapped significantly fewer *D. valens* than did the control (Table 3), suggesting that either system might provide protection for plantation trees from *D. valens* attack. Although the MEC product was slightly better at inhibiting attraction of *D. valens* to synthetic host volatiles, this difference was not significant. Each of the formulations has advantages for pest management, depending on land allocation, human exposure, and management objectives. The bubblecap formulation of verbenone is likely to be especially valuable in situations where transport of a liquid formulation is problematic, whereas the MEC formulation has promise for aerial application in plantations and spot treatments in campgrounds and parks, where unobtrusive treatments may be preferred. Aerial application of verbenone impregnated beads was found to control damage by *D. ponderosae* (Shea et al. 1992), but these authors report that they were unable to duplicate their results in a later study. Miller et al. (1995) summarize the inconsistent results of tests of bead and bubblecap formulations of verbenone for area-wide control of other *Dendroctonus* spp. in forest stands, attributing failures to variable pheromone dispersal and dose-dependent variations in beetle response. Unlike most of its congeners, however, *D. valens* does not usually mass-attack its host trees (Furniss and Carolin 1977), and this behavioral difference has implications for pest management. Both of the formulations tested in this study have promise for a single-tree protection strategy. Stand protection strategies using the MEC are also promising, because the MEC has properties that may render it more effective under operational conditions than bead or bubblecap formulations. For instance, an MEC formulation might adhere better to foliage and bark than do beads, and thus provide a semiochemical plume more appropriate for interruption of host location by *D. valens.* The bubblecap formulation may prove especially valuable in situations, e.g., intensively managed plantations, where the crop has high value and access by road is available. Both formulations provide a potentially effective means for controlling *D. valens* damage while avoiding the use of toxic insecticides.

For the nontarget insects tallied, verbenone functioned mostly as an interruptant to attraction to traps baited with host kairomones (Table 2). In the midseason test (Table 2), verbenone reduced trap catch of cerambycids, buprestids, elaterids, and clerids. Verbenone was behaviorally neutral for raphidiids in midseason tests, but it appeared to attract siricids. In the late season test using both formulations of verbenone (Table 3), none of the differences in trap catches of nontarget insects was significant. It should be noted that in the late season test, as in the midseason test, raphidiids were not attracted to verbenone. The lack of consistent attraction of raphidiids, which are important predators of forest insects (Furniss and Carolin 1977), is reassuring from the standpoint of operational use of verbenone. It is also noteworthy that the secondary buprestid and cerambycid (mostly *Leptura obliterata* Haldeman) borers were repelled by verbenone. Finally, in the midseason test, clerids were repelled by verbenone. Clerids are among the most important natural enemies of *Dendroctonus* bark beetles, but they are generalist predators that consume many other bark beetle species as prey (Furniss and Carolin 1977). The repellency of verbenone to clerids,

Table 3. Late season response of *Dendroctonus valens* and nontarget insects to traps baited with host kairomones, with and without verbenone bubblecaps or microencapsulated verbenone

Treatment	*D. valens*	Cerambycidae	Buprestidae	Raphidiidae
CTR	2.8a (2.0–4.0)	2.5a (1.5–4.1)	0.9a (0.4–2.0)	4.0a (1.8–8.5)
BCP	0.4b (0.16–1.0)	2.7a (1.5–4.0)	0.4a (0.1–1.3)	1.7a (0.5–5.4)
MEC	0.3b (0.10–0.9)	2.5a (1.6–4.3)	0.4a (0.1–1.3)	4.1a (1.9–8.6)

Mean number of insects responding per trap (lower and upper 95% CI); means followed by different letters in a column are significantly different at $\alpha = 0.05$, maximum likelihood test with Bonferroni approach; CTR = Lindgren traps baited with host kairomones (control); BCP = Lindgren traps baited with host kairomones and verbenone bubblecaps; MEC = Lindgren traps baited with host kairomones and microencapsulated verbenone.

then, would conserve potential natural enemies if verbenone were deployed operationally for tree protection. All of the beetle taxa tallied in this study, including representatives from five Coleopteran families (Scolytidae, Elateridae, Cerambycidae, Buprestidae and Cleridae) responded to verbenone as an interruptant. The general repellent effect of verbenone toward all of the beetle groups in this study suggests the potential for repellency to a wider array of Coleoptera. Although verbenone has been shown to synergize attraction in some species of *Dendroctonus* and *Conophthorus* Hopkins spp. at some release rates (Smith et al. 1990, Rappaport et al. 2000), the overwhelming preponderance of data suggests that verbenone functions largely as an interruptant for the Scolytidae and perhaps most Coleoptera.

Pest Management Implications. Our results provide the first semiochemical based option for control of *D. valens.* Our findings that verbenone inhibits host location by *D. valens* and that two release systems are effective in virtually turning off beetle response to baited traps are very promising for the development of operational techniques to reduce or prevent damage by *D. valens,* both in plantations and in sites where individual, high value trees warrant protection. None of the nontarget insect behavioral responses gives rise to concern for operational control programs. There is no evidence that verbenone treatments would disrupt populations of natural enemies nor trigger outbreaks by secondary phloem- or wood-boring insects. It should be noted, however, that this work was conducted on surrogate trees. Further tests using actual plantation trees are necessary to validate our results in terms of crop protection and develop operational methodology.

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